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Effects of prenatal irradiation on fetal, neonate, and young adult murine hemopoiesis

S. R. Weinberg

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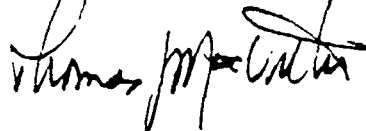
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REVIEWED AND APPROVED



THOMAS J. MACVITTIE, Ph.D.
Chairman
Experimental Hematology Department



LAWRENCE S. MYERS, Ph.D.
Scientific Director



BOBBY R. ADCOCK
COL, MS, USA
Director

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20. ABSTRACT (continued)

marrow hemopoietic progenitor cells. Fluctuations from normal hematopoietic values were greater in the 15 day-old juvenile than in the 9 day-old neonate, commencing with 50 rad. These included peripheral blood parameters and marrow- and spleen-derived erythroid-, granulocytic- and megakaryocytic-progenitor cells. The consequences of prenatal irradiation (150 rad) were evident in the 13 week-old mouse. This was manifested by a reduced spleen cellularity and perturbations in concentrations of hemopoietic progenitor cells in the bone marrow.

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EFFECTS OF PRENATAL IRRADIATION ON FETAL, NEONATE, AND YOUNG ADULT MURINE HEMOPOIESIS

SHEILA R. WEINBERG, PH.D.

Experimental Hematology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814

B6D2F1 mice received cobalt-60 radiation on day 10.5 of gestation at doses of 50 to 300 rad at a dose rate of 40 rad per min. These animals were studied at four selected age periods: (a) day 14.5 of gestation, (b) neonate, (c) juvenile, and (d) 13 week-old adult. Fetal liver cellularity, morphology, and hemopoietic progenitor cell concentration reflected injury after 200 rad. The 15 day-old mouse spleen cellularity was affected more than bone marrow cellularity, but greater radiation injury was reflected by bone marrow hemopoietic progenitor cells. Fluctuations from normal hematopoietic values were greater in the 15 day-old juvenile than in the 9 day-old neonate, commencing with 50 rad. These included peripheral blood parameters and marrow- and spleen-derived erythroid-, granulocytic- and megakaryocytic-progenitor cells. The consequences of prenatal irradiation (150 rad) were evident in the 13 week-old mouse. This was manifested by a reduced spleen cellularity and perturbations in concentrations of hemopoietic progenitor cells in the bone marrow.

Prenatal irradiation, Murine hemopoiesis.

INTRODUCTION

The nature of prenatal pathogenetic consequences induced by low-dose ionizing radiation has been a problem receiving the attention of the medical community^{7,17,19,20,26,29,30,32} and animal model experimentation.^{1,3,8,9,11,14,21,23,25,27,31,33} Increased incidences of exposure to diagnostic and therapeutic radiation as well as environmental radiation hazards have been the main causes for concern. The critical variables at the time of exposure causing the congenital anomalies include the age of fetal development, dose, and dose rate. However, biasing of factors while collecting the information has resulted in a pronounced dichotomy in the literature concerning long-lasting effects of (a) *in utero* or preconception exposure to diagnostic X rays and (b) the atomic bombings of Hiroshima and Nagasaki.^{20,30} Reported anomalies include high incidence of leukemia and other cancers, Down's syndrome, altered sex ratio of offspring, and an elevated death rate of children. Animal experimentation as well as data from clinical studies have documented neurological and skeletal abnormalities in juveniles irradiated during fetal development.^{1,11,17,21,23,27}

In our laboratory we have investigated the hemopoietic perturbations in mice exposed to various doses of total-body gamma radiation (i.e., 50-300 rad of cobalt-60) at 10.5 days of gestation, which is considered the peak of stem cell activity in the extraembryonic loci for blood cell formation.¹⁶ Mice were evaluated for hematopoietic anomalies during their (a) fetal life—day 14.5 of gestation, (b) neonatal life—2-9 days of age, (c) juvenile life—15-19 days of age, and (d) young adulthood—13 weeks of age. In this paper we report the peripheral blood hemogram values and the hemopoietic progenitor cell activity of the blood cell-forming tissues (i.e., bone marrow and spleen) in these groups after exposure to the different doses of irradiation.

METHODS AND MATERIALS

Mice

Groups of virgin female C57Bl/6J mice (10-14 weeks old)* were randomly mated with male DBA/2J mice (10-18 weeks old)* during a period of 24 hr, designated as day 0 of gestation. On day 10.5 of gestation pregnant mice received a bilateral total-body irradiation (TBI) of

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Research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council.

These studies were presented at the 11th Annual Meeting of

the International Society for Experimental Hematology, Baltimore, Maryland, on August 15, 1982.

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*Jackson Labs, Bar Harbor, ME.

either 50, 100, 150, 200, or 300 rad from the Armed Forces Radiobiology Research Institute cobalt-60 gamma radiation source, at a rate of 40 rad per minute. Nonirradiated pregnant mice served as the control group. The assumption was made that the pregnant mice and developing fetuses received the same dose of radiation on day 10.5 of gestation. Thereafter, selected ages studied were fetus at day 14.5 of gestation and after birth ages 2-9 days, 15-19 days, and 13 weeks. All mice were maintained on a diet of food pellets† and acidified water (pH 2.5) made available *ad libitum*. All animals were housed in a temperature-controlled room with a 12 hr light-dark cycle. The data reported for each nonirradiated and irradiated group at the age studied reflect values from 5 to 10 mice in at least three replicate studies per time point.

Cell suspensions

Fetal mice livers were aseptically separated, and cell suspensions were prepared.¹¹ For each of the irradiated and nonirradiated fetal groups, the cellularity, morphology of blood cell elements, and hemopoietic progenitor cell activity were assessed. Both femurs and spleen were removed from 9 day-old neonate, 15 day-old juvenile, and 13 week old young adult mice. The tissues were pooled for each of the irradiated and nonirradiated groups. Prepared cell suspensions were evaluated for tissue cellularity, recognized blood cells, and hemopoietic progenitor cell activity.

Clonogenic assays

Microplasma clot cultures were used to study erythroid burst-forming unit (BFU-E) activity, erythroid colony-forming unit (CFU-E) activity,^{11,14} and megakaryocyte colony-forming cell (MEG-CFC) activity.^{14,15} An extract from anemic sheep plasma was used as the source of erythropoietin (EPO, step III, Connaught Labs, Swiftwater, PA, Lot no. 3023-3, 6.7 units per mg protein). Conditioned medium from a murine myelomonocytic leukemia cell line WEHI-3 (WEHI-3-CM)^{14,15} was added to cultures as the MEG-CFC colony-stimulating factor. Double-layer soft agar cultures¹¹ with pregnant mouse uteri extract (PMUE) as the source of colony-stimulating activity (CSA) were used to study granulocyte-macrophage colony-forming cell (GM-CFC) activity.

Peripheral blood

Peripheral blood samples obtained from neonates by decapitation, or from older mice by cardiac puncture, were pooled for each of the irradiated and nonirradiated groups for determination of hematocrit (Hct) percentage, red blood cell (RBC) counts per mm³, white blood cell (WBC) counts per mm³, and percent differential distribution of circulating blood cell elements.

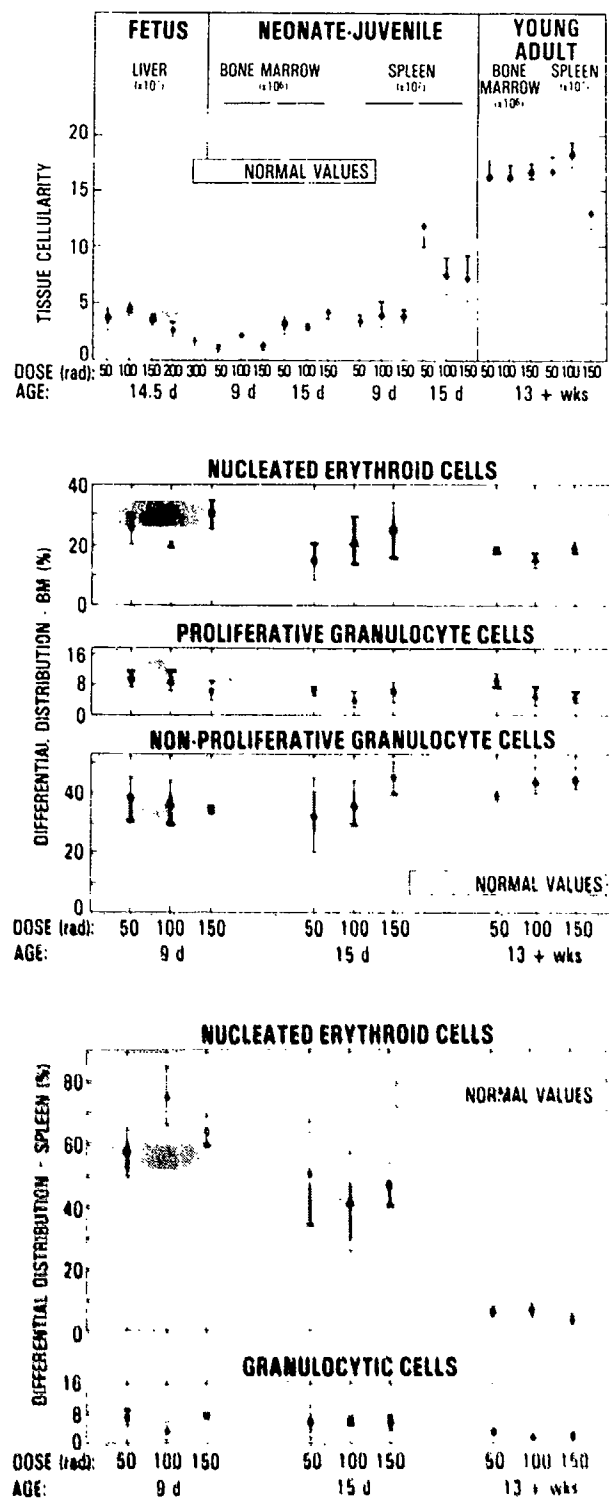


Fig. 1. Fetal liver, neonate, juvenile, and young adult bone marrow and spleen cellularity of normal and prenatally irradiated mice. Bone marrow and spleen differential distribution percent values of normal and prenatally irradiated neonate, juvenile, and young adult mice. Proliferative granulocytic cells include myeloblasts, promyelocytes, and myelocytes. Nonproliferative granulocytic cells include metamyelocytes and mature granulocytes. All values are mean \pm SEM.

†Wayne Lab Blox.

RESULTS

Fetus (day 14.5 of gestation)

At doses of 50 to 200 rad, all 14.5 day-old fetuses were living. Generally 8 fetuses were found per uterus. A 39% increase in aborted day-14.5 fetuses resulted with 300 rad. Most pregnant mice that received a TBI of 200 rad completely aborted their litters (only 2 of 22 pregnant mice delivered litters and the pups did not survive to 15 days). Pups of the 150 rad group were generally smaller in size and had noticeable malformations such as a "runt-like" appearance or hooked tails.

Four days after exposure to 50–150 rad, day-14.5 fetal liver cellularity was not different from normal values. However, exposure to higher doses of 200 and 300 rad on day 10.5 induced sufficient damage, so that cellularity was reduced by 33 and 55%, respectively (Fig. 1).

Although the concentration of the more mature erythroid progenitor cell (CFU-E) appeared normal by 4 days after irradiation (Fig. 2), the concentrations of younger hemopoietic progenitor cells were significantly lower than normal values. For example, following 100 rad, BFU-E were 12% of normal (Fig. 2) and GM-CFC were 50% of normal (Fig. 3).

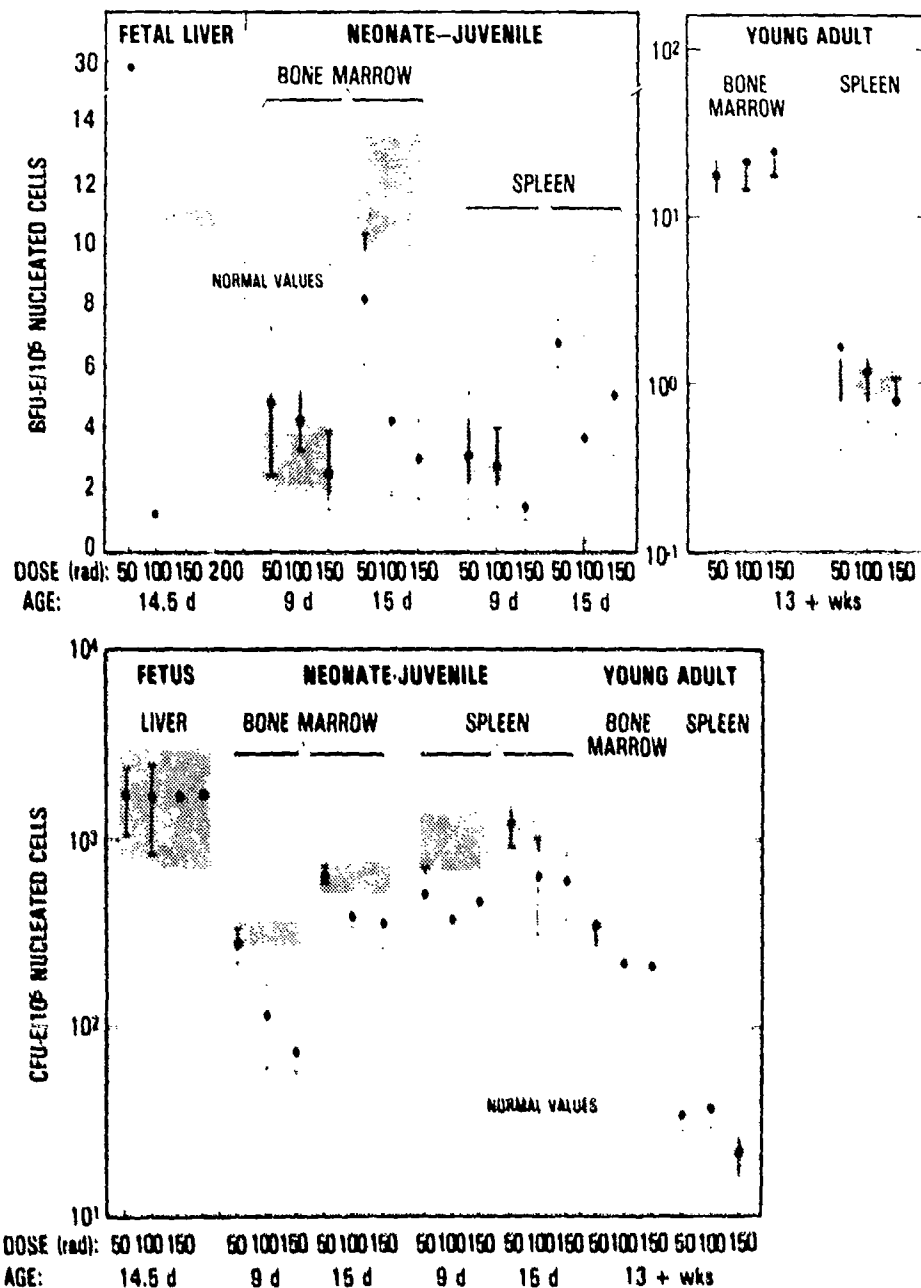


Fig. 2. CFU-E values. Microplasma clot cultures with 0.025 units F-PD per 0.1 ml were harvested after 48 hours. BFU-E values. Microplasma clot cultures with 0.3 units F-PD per 0.1 ml were harvested on day 9. Values are expressed as mean \pm SEM for fetal liver, and bone marrow and spleen from neonate, juvenile, and young adult normal and prenatally irradiated mice.

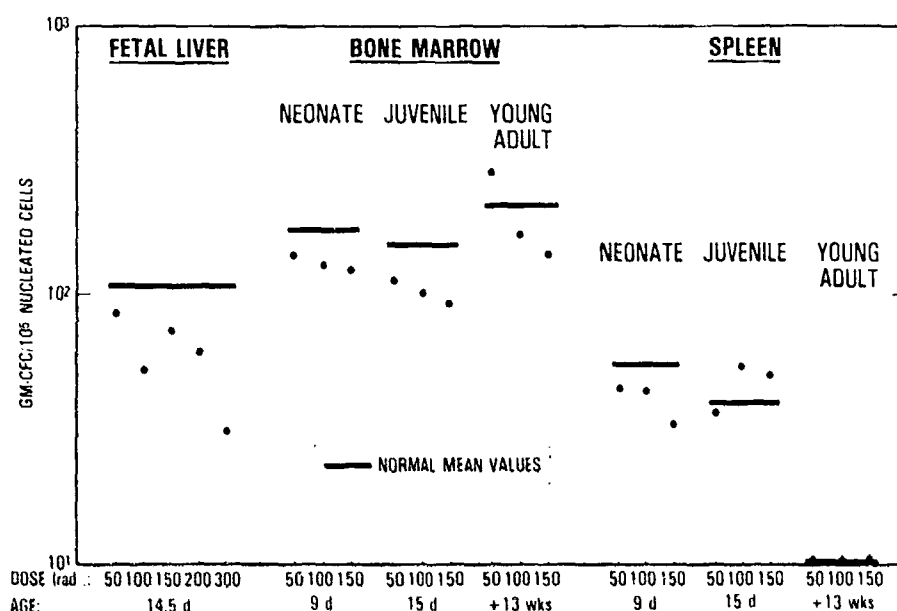


Fig. 3. GM-CFC values: Double-layer soft agar cultures with PMUE as a source of CSA. Values are expressed as the mean GM-CFC for fetal liver as well as bone marrow and spleen from neonate, juvenile, and young adult normal and prenatally irradiated mice.

Neonate (9 days old) and juvenile (15 days old)

Bone marrow cellularity of the neonate and juvenile were not affected by prenatal radiation exposure to doses of 50-150 rad (Fig. 1). However, the percent marrow recognizable proliferative granulocytes (myeloblasts, promyelocytes, and myelocytes) of irradiated 9 day-old mice were lower than values for normal mice (Fig. 1). Fluctuations from normal marrow cell composition were not observed in 15 day-old mice.

Although spleen tissue cellularity was slightly lower than control values (Fig. 1), the differential distribution of recognizable hemopoietic cells (Fig. 1) reflected greater fluctuation from normal with each ionizing radiation dose. Compared to the normal spleen, nucleated erythroid cells were higher in irradiated 9 and 15 day-old mice, and granulocytic cells were lower only in the irradiated 9 day-old mice.

Although the marrow and spleen CFU-E (Fig. 2) of the irradiated (<100 rad) 9 and 15 day-old mice were lower than normal, the BFU-E of only the irradiated 15 day-old mice were lower than normal (Fig. 2). GM-CFC derived only from irradiated 9 and 15 day bone marrow were reduced (Fig. 3). A peripheral blood erythrocytopenia and leukocytosis (Fig. 4) in the control groups were apparently caused by the low 9 day-old marrow and the spleen BFU-E and CFU-E as well as the high spleen GM-CFC. The elevated values of the 15 day-old spleen nucleated erythrocytic cells, BFU-E, and CFU-E appeared to resolve the peripheral blood erythrocytopenia in the 19 day-old mice. Prenatal irradiation resulted in further significant decreases in the peripheral blood erythron indices to below the anemia levels of control neonate mice (Fig. 4). However, with each higher dose of exposure, the counts increased above the previous value.

The reverse situation occurred with the peripheral blood WBC counts (Fig. 4); each higher dose resulted in a further decrease in WBC counts in the neonates to below previous values.

The concentration of bone marrow MEG-CFC from irradiated 9 day-old mice (<7.3 MEG-CFC per 10^5 cells) was higher than the nonirradiated (3.2 MEG-CFC per 10^5 cells). The irradiated 15 day values (<2.8 MEG-CFC per 10^5 cells) were lower than the normal values (5.2 MEG-CFC per 10^5 cells). Spleen-derived MEG-CFC from irradiated 9 day-old mice (2.8-3.3 MEG-CFC per 10^5 cells) were similar to those of the normal (2.6 MEG-CFC per 10^5 cells). Values for irradiated (100 and 150 rad) 15 day-old mice were lower (0.66 and 1.46 MEG-CFC per 10^5 cells, respectively) than MEG-CFC from the same age nonirradiated and 50 rad groups (2.5 and 4.2 MEG-CFC per 10^5 cells, respectively).

Young adult (13 weeks old)

The difference observed between cellularity numbers of irradiated and nonirradiated young adult spleen was greater than the differences in neonate and juvenile mice (e.g., irradiation with <50 rad resulted in a 28% decrease in the 15 day-old mouse but a 30% decrease in the young adult) (Fig. 1). Bone marrow cellularity of the irradiated groups of mice were within the range of values for nonirradiated mice.

No significant sustained effects of prenatal irradiation were observed in young adult marrow and spleen concentrations of BFU-E (Fig. 2). Although irradiated (<100 rad) young adult medulla-derived CFU-E were lower than values for control mice of the same age, the numbers of irradiated (50 and 100 rad) spleen-derived CFU-E

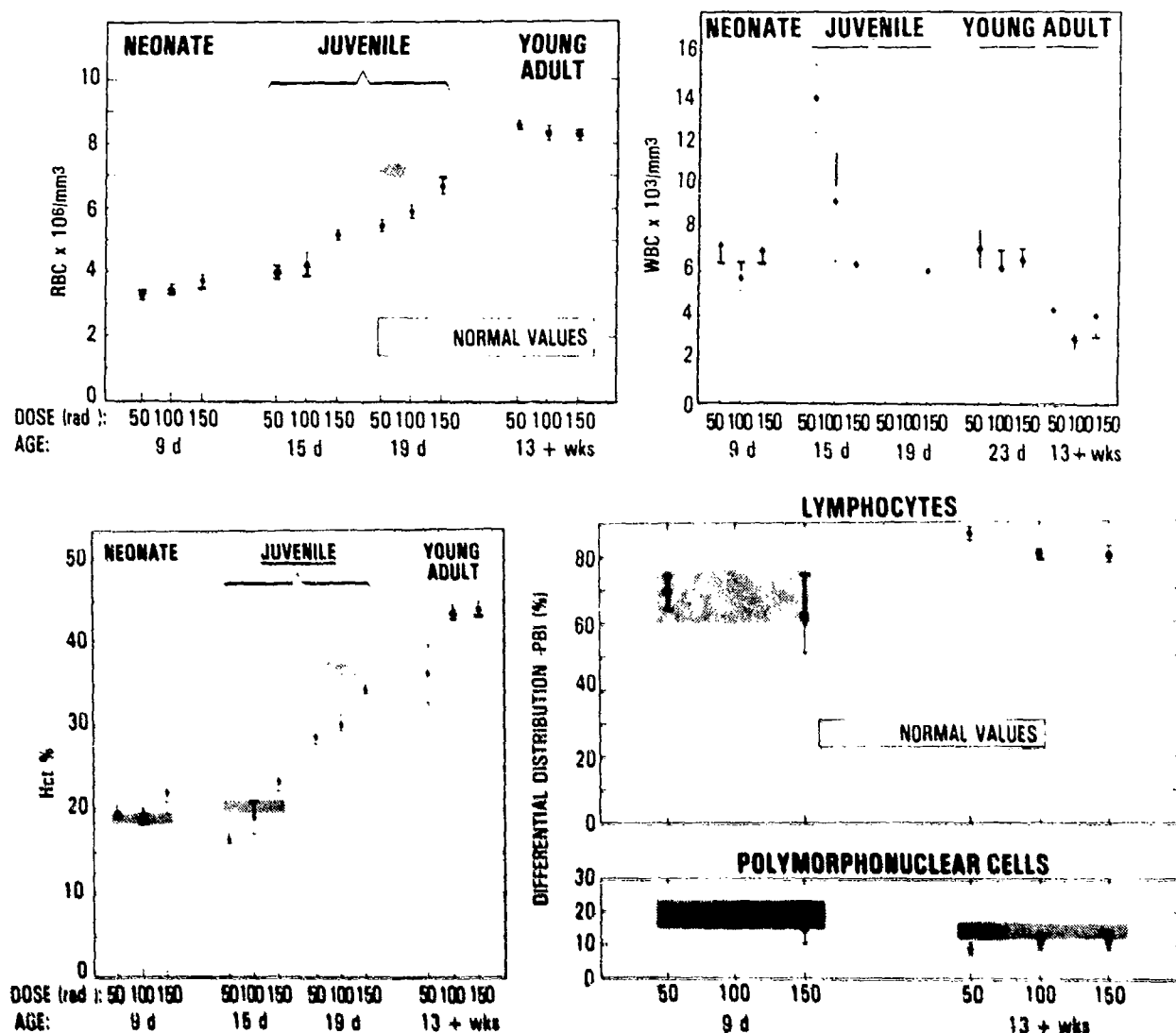


Fig. 4. Peripheral blood red blood cell counts (RBC) per mm³, hematocrite (Hct) percent, white blood cell counts (WBC) per mm³, and differential distribution percent values of normal and prenatally irradiated mice. All values expressed as mean \pm SEM.

were higher than those of normal mice (Fig. 2). Modulations from the normal young adult were evident in peripheral blood erythron and leukocyte indices of the irradiated groups (Fig. 4). However, decreases in the concentration of marrow-derived GM-CFC from irradiated young adult groups (Fig. 3) and marrow- and spleen-recoizable proliferative granulocytic cells (Fig. 1) could not have accounted for the observed peripheral blood leukocytosis (Fig. 4). Examination of the adult peripheral blood cell elements confirmed this assumption. The percent circulating granulocytes were lower than for normal mice, and the percent lymphocytes were increased above normal values (Fig. 4). The effects of prenatal irradiation on adult marrow MEG-CFC activity (Fig. 5) were similar to those on the CFU-E and GM-CFC activity. On the other hand, the irradiated adult spleen MEG-CFC values were within normal values, and were similar to spleen BFC-E and GM-CFC activity but unlike CFU-E activity.

DISCUSSION

This paper reports the long-lasting effects of prenatal irradiation on murine hemopoiesis of the fetus, neonate, juvenile, and young adult mouse. It is not known whether the fluctuations observed in tissue cellularity and hemopoietic parameters of the peripheral blood, bone marrow, and spleen from prenatally irradiated mice are a result of damage to the migrating stem cells from the yolk sac on day 10.5 of gestation during the stage of active organogenesis,^{16,17,18} or due to random cell damage that is of a sufficient degree throughout the blood cell-forming tissues to result in defects in the regulatory mechanisms of hemocytopenesis.¹⁹

Day 14.5 fetal liver was selected to reflect radiation damage. Since the day 14.5 tissue is more than 60% hematopoietically active,^{17,18} any fluctuations from the normal hemogram following prenatal exposure to irradiation would indicate the degree of damage and the degree

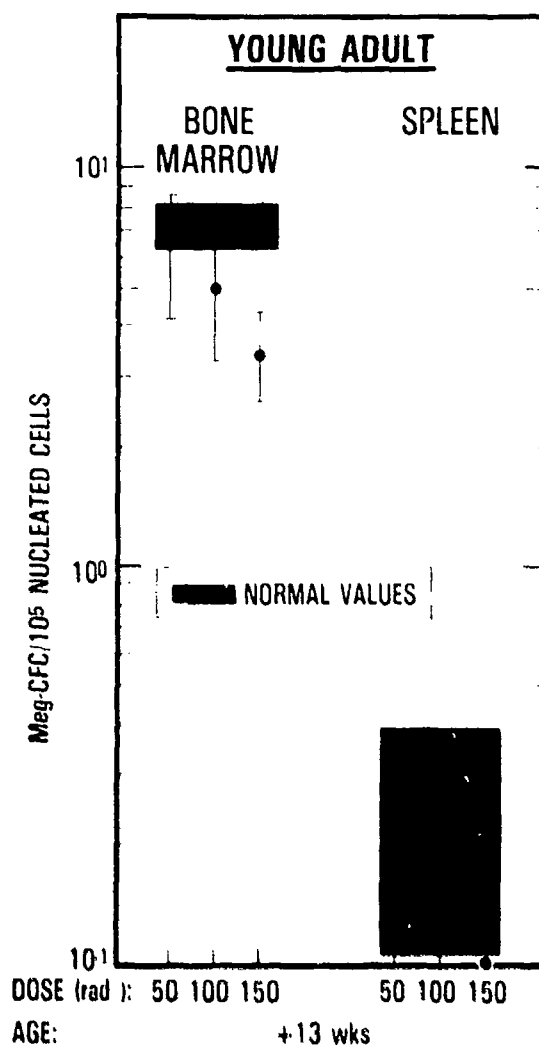


Fig. 5. MEG-CFC values. Microplasma clot cultures with WFH-3CM (five-fold concentration, 10^7 v/v), harvested on day 7. Normal (mean \pm SEM) and prenatally irradiated young adult bone marrow (mean \pm SEM) and spleen (mean) values.

of recovery ability of this primary site of hemopoiesis. The results showed fetal liver granulopoiesis to be more radiosensitive to injury compared to erythropoiesis. However, the fetal liver appears to have a greater potential for erythropoiesis recovery. This is not an unexpected finding since the day 14.5 fetal liver is greater than 80% erythropoietically active, as indicated by cyto-spin smear preparations of tissue cell suspensions and the high concentration of CFU-E per 10^5 cells compared to values of the adult bone marrow and spleen.

Our observations showed the spleen of the 9 day-old neonate to be at maximal erythropoietic activity with a high relative number of nucleated erythroid cells and CFU-E. The bone marrow at this age has a low concentration of BFU-E and CFU-E. Others have also reported the 9 day-old spleen as the primary site of erythropoietic activity.^{13,24} The dramatic increase in the 15 day-old mouse marrow BFU-E and CFU-E concentrations, spleen

BFU-E, and continued high CFU-E concentrations resolved the neonatal peripheral blood erythrocytopenia reflected in the values of the 19 day-old mouse RBC counts and Het percentages (Fig. 4). Prenatal radiation-induced decreases in 19 day-old mouse medulla- and spleen-derived BFU-E, CFU-E and GM-CFC were reflected in the 19 day-old peripheral blood values for RBC, Het, and WBC.

Our reported differences in values of the hemopoietic progenitor cells derived from mouse fetal liver and young adult tissues following prenatal irradiation exposure are supported by other investigators.^{12,28} The highly proliferative fetal cells at day 10-13 of gestation did not appear to exhibit the same extent of radiation damage as the neonate, juvenile, or adult cells. In fact, the expression of injury may be delayed until a later age. During midstage of murine gestation (a stage of active organogenesis, when rapid development of the hemopoietic system is occurring), the fetal tissues may experience radiation injury of a sufficient degree. Although cell damage may be randomly distributed, cell-to-cell interactions in the hemopoietic microenvironment that regulate hemocytopenesis may be permanently disrupted. An example of the latent effects of radiation injury may be the higher incidence in the onset of childhood leukemia in children who have been exposed *in utero* to radiation.^{29,30}

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